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31P-Edited Diffusion-Ordered 1H NMR Spectroscopy for the Isolation and Identification of Organophosphorus Compounds Related to Chemical Weapons Agents and their Degradation Products

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^{31}P-edited diffusion-ordered ^{1}H NMR spectroscopy for the isolation and identification of organophosphorus compounds related to chemical weapons agents and their degradation products

Brian P. Mayer, Carlos A. Valdez, Saphon Hok, Sarah C. Chinn, Bradley R. Hart*

Forensic Science Center, Lawrence Livermore National Laboratory, 7000 East Ave, Livermore, CA 94550

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Abstract.

Organophosphorus compounds constitute a large class of molecules, many of them pesticides, flame-retardants, biologically relevant molecules, and chemical weapons agents (CWAs). The detection and identification of organophosphorus molecules, particularly in the cases of pesticides and CWAs are paramount to the verification of international treaties by various organizations. To that end, novel analytical methodologies that can provide additional support to traditional analyses are important for unambiguous identification of these compounds. We have developed an NMR

method that selectively edits for organophosphorus compounds via ^{31}P-^{1}H heteronuclear single quantum correlation (HSQC) and provides an additional chromatographic-like separation based on self-diffusivities of the individual species via ^{1}H diffusion ordered spectroscopy (DOSY): ^{31}P-^{1}H HSQC-DOSY. The technique is first validated using the CWA VX (a phosphonothioate) by traditional two-dimensional DOSY spectra. We then extend this technique to a complex mixture of VX degradation products and identify all the main phosphorus-containing byproducts generated after exposure to a zinc-cyclen organometallic homogeneous catalyst.

Introduction.

Though emphasizing the criticality of the destruction of existing chemical-based weapons since the inception of the Chemical Weapons Convention (CWC) in 1997, the CWC's core mission has made the development of methodologies to assess, to surveil, and to analyze the presence of chemical weapons agents (CWAs) and their associated synthesis and degradation byproducts an equally important enterprise. A large majority of CWAs scheduled by the Organization for the Prohibition of Chemical Weapons (OPCW) are or are related to organophosphorus compounds with notable examples that include VX, soman, and sarin. From a structural perspective, these compounds are generally characterized by the presence of a phosphorus atom serving as an anchor point to which alkyl groups (C_1-C_{10}) may be bonded to directly or indirectly (i.e. via the bridging intermediacy of a heteroatom such as O, S, or N).

Samples relating to OPCW inspections and proficiency examinations represent a wide variety of matrices that may include aqueous or organic solutions, various soil types, or

viscous/oily residues. Their analysis is typically characterized by sample preparatory methods that make the mixture amenable for chromatographic analyses combined with mass spectrometry (e.g. GC/MS and LC/MS). However, the correct identification of an analyte of interest can become a challenging task due to overwhelming interferences arising from the chemical compositions of the sample and the surrounding matrix. For this reason, the use of several chromatographic methods in parallel to attain a successful analysis is not only common but often a necessary approach to take when dealing with these types of samples.

In the past twenty years, nuclear magnetic resonance (NMR) spectroscopy has gained considerable status as a powerful tool in the analysis and identification of toxic organophosphorus-based compounds. The high natural abundance and receptivity of both ^{1}H and ^{31}P nuclei make NMR an ideal tool for the analysis of phosphorus-containing CWAs. These natural properties when coupled to the advances in modern hardware technology, particularly in the field of cryoprobes, allow for the detection of relatively low concentrations (~1 ppm) of analytes within reasonable experimental time frames.

Samples originating from actual, real world scenarios often contain an unidentifiably large number of components that effectively prohibit the ultimate utility of ^{1}H NMR, as signals related to phosphorus-containing CWAs will very likely be buried under or masked by those of solvents, dominant impurities, etc. Heteronuclear NMR (^{31}P or ^{19}F in the cases of sarin and soman, for example) offers several solutions to this complex scenario in that 1) typically fewer resonances will be encountered 2) heteronuclei generally enjoy wider native chemical shift ranges than that of ^{1}H nuclei, and 3) splitting patterns due to J-coupling provide some limited insight into the structural details of the

alkyl side chains. Despite these advantages, it is often undesirable to directly detect signals from phosphorus and fluorine, as they are not as sensitive as proton nuclei in an absolute sense. Therefore, proton-heteronucleus correlation experiments that detect on the proton channel but contain information about couplings to other nuclei have become critical in CWA analysis by NMR. In particular one- and two-dimensional ^{1}H-^{31}P heteronuclear single quantum coherence (1D/2D ^{1}H-^{31}P HSQC) is a highly effective experiment that spectrally edits out all proton signals not directly involved in phosphorous-containing compounds due to lack of ^{1}H-^{31}P J-coupling. This approach in combination with total correlation spectroscopy sequences (e.g., HSQC-TOCSY) often allows for unequivocal structural elucidation of alkyl and hetero-alkyl side chains in the analyte of interest.[1-3]

The majority of HSQC-based NMR techniques employed for studying phosphorus-containing CWAs have been two-dimensional correlation methods. That is, the information contained in both dimensions is *spectral* in nature, where, for example, cross peaks indicate J-coupled protons and phosphorus nuclei. Considering the relatively narrow proton chemical shift range, mixtures containing even low numbers of phosphorus-containing compounds can render HSQC-edited proton spectra difficult to interpret, particularly for one-dimensional data. For this reason, an alternative approach for the analysis of complex proton spectra has centered around the concept of diffusion ordered spectroscopy (DOSY).[4, 5] In this technique, the detected proton signals are separated based on the self-diffusion coefficients of individual molecules. DOSY then amounts essentially to a chromatographic-type separation, where diffusivity appears along the traditional F_1 (or indirect) dimension of the 2D spectrum as opposed to

chemical shift. In combination with the HSQC methods described above, one can in principle edit out all proton signals but those J-coupled to phosphorus nuclei and establish a direct relationship between the remaining resonances and their respective diffusivities.

Several authors have reported on the design and practical application of HSQC-DOSY and related techniques in the past, but this work has only focused on carbon (^{13}C) as the heteronucleus in question.[6-8] For example, McLachlan and coworkers[8] detail a constant time gradient 3D HQSC-DOSY variant to distinguish between chemical cognates, rutin and quercetin, that exhibit resonances with very similar chemical shifts in both the carbon and proton dimensions. Additionally, while various groups have used ^{31}P DOSY in the past for various applications[9-12], the combination of ^{1}H DOSY *and* ^{31}P NMR heteronuclear correlation editing (via HSQC, for example) has not been addressed. In this work, we demonstrate ^{1}H-^{31}P HSQC-edited ^{1}H DOSY for identifying phosphorous-containing CWAs from complex, proton-rich NMR spectra. Diffusion constants obtained via this route will be validated against traditional ^{1}H DOSY spectra that have not been edited via HSQC. We will also consider the degradation products of VX, using the HSQC-DOSY sequence to not only isolate molecules containing organophosphorous groups but to identify unknown degradation products.

Sample preparation.

VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate, see inset Figure 1) was synthesized at LLNL (see safety note) and was purified via distillation (~97% purity by ^{31}P, ^{1}H NMR and GC-MS). Deuterated chloroform ($CDCl_3$, 99.96 atom % D,

0.03 (v/v) TMS) and deuterium oxide (D_2O, 99.9 atom % D) were purchased from Sigma-Aldrich (St Louis, MO.) and used as received for the preparation of the NMR samples. The preparation of the VX sample was done in enough volume of solvent (typically between 400-550 μL) to yield a final concentration of roughly 1000 ppm VX. For all experiments, the NMR tubes were capped, tightly sealed, and kept refrigerated prior to usage in order to prevent volatiles from evaporating significantly.

We also carried out a VX degradation experiment involving a base-induced process. This base-induced degradation of VX was carried out by dissolving VX (neat oil, 1.1 mg, 4.1 μmol) in D_2O (300 μL) in a 5 mm NMR tube followed by the sequential addition of a 0.39 mM D_2O solution of hexamethylphosphoramide (HMPA internal standard) (100 μL, 0.7 μL, 4.0 μmol) and a 20 mM D_2O solution of Zn^{2+}-cyclen catalyst (30 μL, 0.6 μmol, ~15 mol% to VX). The NMR tube was capped, sealed, and gently vortexed to assure proper mixing of the components. HMPA was chosen as an internal standard for its excellent water solubility, its well-defined ^{31}P and ^{1}H NMR resonances (29.8 ppm and 2.56 ppm respectively), and its stability to the conditions under which the degradation experiments were executed at (i.e. catalyst concentration and the pH range 7.0-9.1). The catalyst used in these experiments was Zn^{2+}-cyclen as its perchlorate salt and was prepared as described previously.[13] The specifics behind the choice of this catalyst and its relevant degradation chemistries will be discussed in detail in an upcoming publication.

Safety note: VX is a highly toxic compound that can harm exposed individuals at extremely small doses (e.g. dermal LD_{50} < 0.1 mg/kg[14]). Only properly trained personnel, in a certified laboratory possessing the adequate equipment to carry out their

synthesis and subsequent purification, should handle highly toxic chemical warfare agents. The Forensic Science Center at Lawrence Livermore National Laboratory (LLNL) has the authority and capability to synthesize and handle small quantities of VX through its accreditation as a United States Designated Laboratory for the Organization for the Prohibition of Chemical Weapons, which performs monitoring for verification of international treaties that ban chemical weapons.[15] Proper protective personal equipment (PPE) should be worn at all times which include lab coats, safety glasses, butyl-based gloves with nitrile gloves underneath to provide further protection and a face shield. The handling and preparation of the NMR samples should be conducted inside a well-ventilated and certified chemical fume hood.

NMR experimental.

All experiments were executed on a Bruker Avance III 600 MHz instrument equipped with a Bruker TCI 5mm cryoprobe (Bruker Biospin, Billerica, MA) at 30.0 ± 0.1°C. The basic pulse sequences used were canned experiments provided by the manufacturer (see below).

In addition to its low volatility and its similarity to a wide range of other organophosphorous compounds (e.g. pesticides), VX was chosen due to its favorable NMR line shape characteristics. Many phosphates, phosphonates and other phosphorus-containing molecules containing alkoxy side groups off the central phosphorus nucleus (e.g. sarin, soman) are HH□P systems from an NMR perspective. The remote H□ proton affects the HSQC signal, generating distorted, weak peaks despite $^{4}J_{H\square P} \approx 0$ due to antiphase terms that evolve during the INEPT transfer periods.[3] As also discussed, a Q3

Gaussian cascade pulse was shown to be an effective 180° ^{1}H refocusing pulse, as J_{HP} heteronuclear scalar coupling does not evolve during the application of the pulse. More importantly, the homonuclear coupling is refocused by this selective 180° pulse, and antiphase terms otherwise modulating the detectable magnetization are removed.

In the case of VX, the methyl group directly bound to the phosphorus nucleus dominates the evolution of the detectable magnetization, and little antiphase character is observed in the line shape. Therefore the present experiments require no band-selectivity, as the direct phosphorus-methyl bond yields a strong, in-phase HSQC peak (ca. 1.7 ppm, isolated HP system) when the experiment is optimized for the $^{2}J_{HP} \approx 15.8$ Hz coupling ($\Delta = 1/(2J_{HP})$). Conventional 1D ^{1}H-^{31}P HSQC spectra were obtained using the *hsqcgpnd1d* sequence provided by the hardware manufacturer. The proton and phosphorus carrier frequencies were 4.53 and 30.00, respectively; and the proton spectral window was 10.08 ppm. The nominal ^{1}H and ^{31}P excitation pulse widths were 15.25 and 9.00 μsec, respectively; and the recycle delay was set at 5 seconds. For the conventional DOSY experiment (Bruker sequence *ledbpgp2s*), 32 gradient strengths were selected between 2% and 95% of the maximum pulsed gradient strength of 5.3 G cm^{-1} A^{-1}. 16 scans were taken per gradient strength for a total experimental time of approximately 45 minutes. The data were processed using the commercial software provided by the manufacturer. Considering the nature of the sample and the relatively well-resolved spectra, single component data fitting was determined to be satisfactory (vis-à-vis more complicated inverse Laplace algorithms) for all analyses.

Two versions of the HSQC-DOSY pulse sequence were created for the present work. The first resulted from a simply appending on the *ledbpgp2s* sequence to the end of the

hsqcgpnd1d experiment with minor modifications to the phase cycling of the pulses. The second sequence was identical to the first except for a low power presaturation pulse (1 s, 0.154 mW) prior to the first excitation pulse on the ^{1}H channel. This experiment is shown schematically along with phase cycling in **Figure 1**. It is important to point out the non-traditional receiver phasing in these experiments. In order suppress the effects of undesirable terms in the detectable magnetization (in a product operator sense) the receiver phases of the nominal HSQC and DOSY pulse sequences were added together in a cyclical fashion.

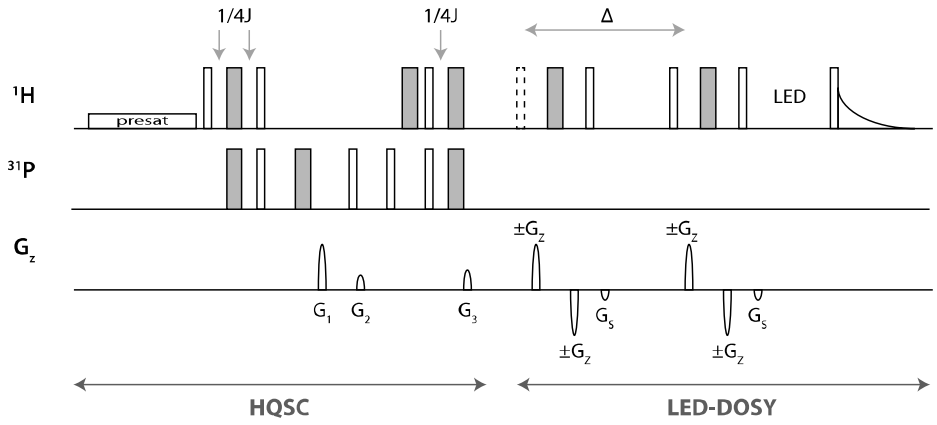

Figure 1. ^{1}H-^{31}P HSQC-DOSY experiment with solvent presaturation. Thin white rectangles and thick grey rectangles correspond to 90° and 180° pulses, respectively. During the excitation/reconversion segments of the double INEPT HSQC portion, J_{HP} = 15.8 Hz was used to calculate the interpulse delay. The dashed 90° pulse traditionally used to initiate the DOSY experiment is omitted due to the presence of the preceding pulses (and therefore ignored from the phase table below). Δ corresponds to the time between the center of the two positive gradient pulses, $+G_Z$; and δ is twice the duration of one positive gradient pulse, $+2G_Z$. Gradient pulses are given in the bottom row: G_1 =

80%, G_2 = 30%, G_3 = 32.4%, G_s = -13.17%, and G_Z was stepped from 2% to 95% of the maximum gradient strength of 5.3 G cm^{-1} A^{-1}. The phase cycling is here given beginning with all proton pulses *in order left-to-right* (dashed pulse omitted) then following with the phases of the phosphorus and receiver channels. ^{1}H: $\{x\}_{presat}$, $\{x\}$, $\{x\}$, $\{y\}$, $\{x\ x\ -x\ -x\}$, $\{y\}$, $\{x\}$, $\{x\}$, $\{x\ x\ -x\ -x\}$, $\{x\ x\ x\ x\ -x\ -x\ -x\ -x\}$, $\{x\}$, $\{x\ -x\ x\ -x\ -x\ x\ -x\ x\}$, $\{x\ x\ x\ x\ -x\ -x\ -x\ -x\}$; ^{31}P: $\{x\}$, $\{y\ y\ y\ y\ -y\ -y\ -y\ -y\}$, $\{x\}$, $\{-y\}$, $\{x\}$, $\{x\ -x\}$, $\{x\}$; acquisition: $\{x\ x\ -x\ -x\ x\ x\ -x\ -x\}$.

Results and Discussion.

Distilled VX.

Figure 2a shows the one-dimensional proton spectrum of the synthesized VX sample. VX peaks and additional peaks presumably from synthesis solvents are both identified on the spectrum. Because the synthesis results were purified by distillation it is not surprising to have a relatively clean, well-resolved NMR spectrum. This observation will be exploited when testing the HSQC-DOSY experiment against results obtained by the more routine, manufacturer supplied DOSY technique. In addition, **Figure 2b** shows the 1D ^{1}H-^{31}P HSQC spectrum of the same sample showing the CH_3-P doublet associated with VX.

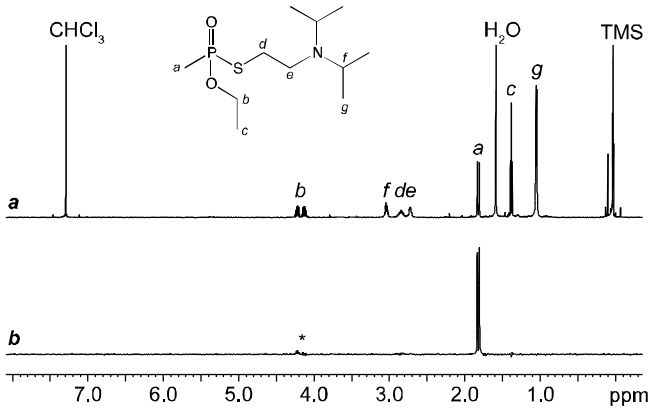

Figure 2. a) ^{1}H spectrum of the distilled VX sample. Solvent impurities identified, and the structure of VX and resonance assignments given. b) 1D ^{1}H-^{31}P HSQC (J = 15 Hz) spectrum showing CH_3-P doublet. Asterisk represents some small anti-phase signal from the ethoxy methylene protons.

Figure 3 shows the results of the DOSY experiment using the *ledbpgp2s* sequence. Note that the raw NMR data has been phased and baseline corrected before execution of the DOSY analysis. A brief look at the figure reveals that seven proton signals share a common diffusivity and can therefore be assigned to the protons of VX (with confirmation 1D/2D NMR and library spectra). At the current experimental conditions (30°C in $CDCl_3$) the diffusivity of VX is $D_{VX} = 1.37 \pm 0.04 \times 10^{-9}$ m^2/s. Note that the linewidth of the fully processed DOSY spectrum reflects the uncertainty in the derived diffusivity. The three remaining peaks belong to tetramethylsilane, $CHCl_3$, and residual water, and their diffusivities are $3.23 \pm 0.05 \times 10^{-9}$ m^2/s, $3.265 \pm 0.12 \times 10^{-9}$ m^2/s, and $6.93 \pm 0.10 \times 10^{-9}$ m^2/s, respectively. These results were then used to validate the HSQC-DOSY sequence.

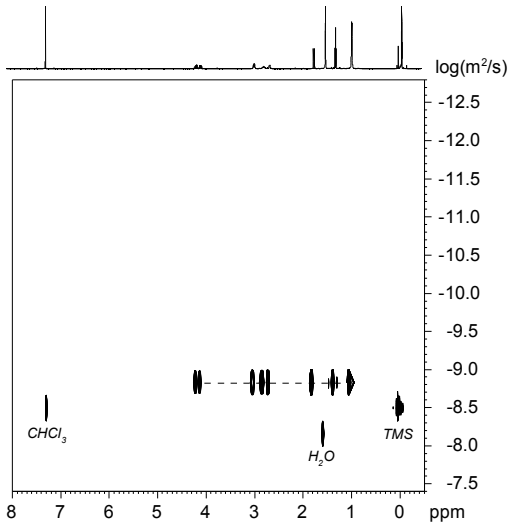

Figure 3. Traditional 2D DOSY plot using *ledbpgp2s* sequence showing log(*D*) on the y-axis. The solvent peaks have been identified explicitly, and the VX peaks have been highlighted with a dashed line. From this plot D_{VX} was determined to be $1.37 \pm 0.04 \times 10^{-9}$ m^2/s.

Figure 4 shows the results for the ^{31}P-edited HSQC-DOSY experiment for the identical distilled VX sample used above. Shown is the peak intensity for the methyl doublet as a function of gradient strength. Because VX is the sole phosphorus-containing molecule in the sample we only observed a single peak in the HSQC-DOSY spectrum (recall **Figure 2b)**. These data were fit to the traditional expression valid for bipolar, simulated echo-type DOSY experiment given as:

$$A(z, \Delta + \delta) = I_0 \exp[-\gamma^2 g(z)^2 \delta^2 (\Delta - \delta/3)] \qquad \text{[eq. 1]}$$

where I_0 is the initial intensity (ca. unity), D is the diffusivity, γ is the proton gyromagnetic ratio, $g(z)$ is the gradient strength (independent variable), and Δ and δ are the durations discussed in **Figure 1**. The data were fit using the commercially available

software package Mathematica (Wolfram, IL, USA). The results from the distilled VX analysis using the HSQC-DOSY sequence gave $D_{VX} = 1.32 \pm 0.05 \times 10^{-9}$ m^2/s, which agrees quite well (within 5%) with the full, unedited DOSY sequence (*vide supra*). Now that the HSQC-DOSY sequence has been confirmed to give identical results to a traditional DOSY experiment, we now turn our focus to applying this technique to a more complex system: VX degradation and resultant product identification.

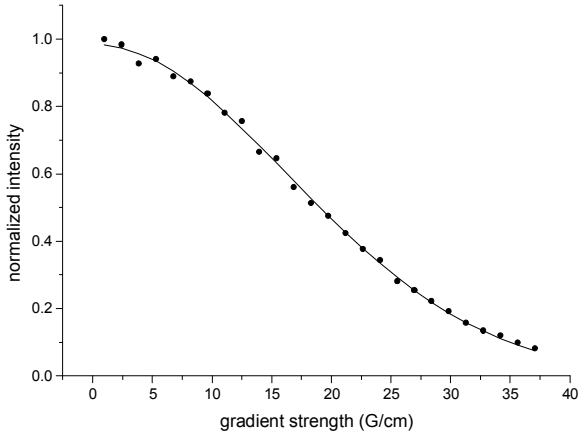

Figure 4. HSQC-DOSY decay for distilled VX solution (in $CDCl_3$, 30°C) as a function of gradient strength (filled circles). The curve is a nonlinear least-squares fit to the NMR data using Equation 1.

VX degradation and product identification.

Assessing the efficacy of organic or inorganic small molecules towards degrading CWAs not only involves confirming the destruction of the target analyte but also entails the identification of the resultant degradation products. This is of particular importance for VX where, depending on the decontamination conditions, these degradation products can be just as toxic in a physiological sense then the parent molecule itself. For example,

under neutral to alkaline conditions (pH = 7–10), P-O bond cleavage dominates producing ethanol and EA2192 (*S*-(2-diisopropylaminoethyl) methylphosphonothioate), a chemical of similar toxicity and anticholinesterase properties (intravenous LD_{50} between 0.24-0.825 times that of VX depending on species).[16] Identifying, then, the products of CWA degradation becomes quite important because there exists a strong motivation to develop decontamination strategies that obviate the generation of such toxic byproducts.

Figure 5 shows the ^1H spectrum of the degraded VX sample. Note that there are a significant number of additional peaks as compared to the fresh VX sample considered previously. The pattern of peaks suggests some VX-like byproducts and the appearance of multiple doublets with J-couplings on the order of 10-15 Hz suggests several new CH_3-P containing species. The goal of this work is to apply the HSQC-DOSY sequence to identify the number and identities of the primary degradation products.

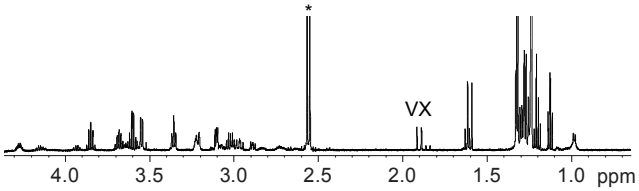

Figure 5. ^1H spectrum of the degraded VX sample. Note the complexity of the spectrum, particularly between 2.5 and 4.0 ppm and at approximately 1.25 ppm. The VX methyl doublet has been labeled, while an asterisk denotes the HMPA methyl doublet.

Because of the significant residual water signal in the NMR spectrum the pulse sequence prefaced with a low power presaturation pulse was used. The DOSY experiment performed was identical to that of the distilled VX sample and the data

obtained was similar in form to those in **Figure 4**. The DOSY decay curves were again analyzed using a nonlinear least-squares approach to yield the diffusivities of all major phosphorus-containing compounds. The goodness of fit is shown for three of the four peaks in **Figure 6**; note that the x-axis is shown as the square of the gradient strength and the y-axis is logarithmic to show linearity of the data. The regression results are given in **Table 1**.

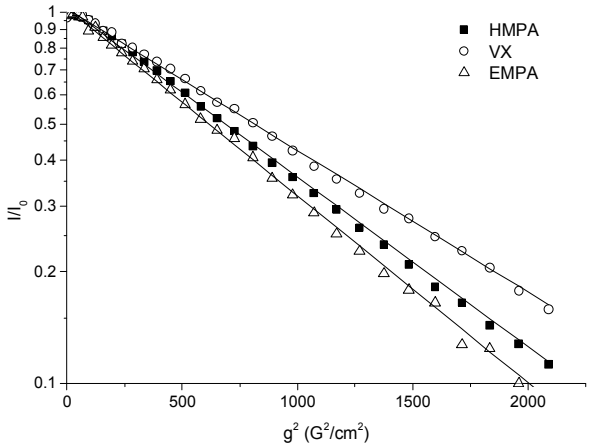

Figure 6. HSQC-DOSY decay curves for HMPA (filled squares), VX (open circles), and EMPA (open triangles). Note that these data are plotted differently than **Figure 4**; they have been 'linearized' by plotting the intensity logarithmically against the square of the gradient strength.

Peak chemical shift (ppm)	Compound Identity	D ($\times 10^{-10}$ m^2/s)	I_0
2.610	HMPA	7.45 ± 0.09	1.03 ± 0.01
1.901	VX	6.22 ± 0.08	1.02 ± 0.01
1.601	--[a]	6.39 ± 0.12	1.02 ± 0.01
1.218	--[a]	8.22 ± 0.10	1.02 ± 0.01

Table 1. Regression results from HSQC-DOSY data of the degraded VX solution. Four major phosphorus-containing compounds were easily measured. Given are the diffusivities and intensities for the various chemical shifts. HMPA and VX resonances have been identified. [a]Additional discussion of compound identities in text.

Because we are using a cyclen-Zn^{2+} catalyst for which the active degradation specie is a hydroxyl ion (HO^-), the aqueous VX solution will be slightly basic. It is well established that between pH 7 to 10 there exists a competition between P-O and P-S bond cleavage.[16] We would therefore expect that the resultant P-containing degradation products are ethyl methylphosphonic acid (EMPA, from P-S cleavage) and EA2192 (from P-O cleavage). Because of possible complexity of the ^1H spectrum, the low amounts of degradation products produced, and the fact that we can only detect CH_3-P doublets in the case of VX, distinguishing easily between these two products may prove difficult. HSQC-DOSY data can yield considerable insight into the nature of the compounds at 1.60 and 1.21 ppm.

The Stokes-Einstein relationship is often invoked to provide correlations between molecular size and weight to diffusivities. Under various assumptions, the smaller the molecule (i.e., the smaller its molecular weight) the faster it should diffuse in a given medium. Looking at the diffusivities in **Table 1**, one would then conclude the peak at 1.218 ppm with the largest diffusivity belongs to EMPA, as it is the suspected product with the lowest molecular weight. Library spectra and two-dimensional NMR techniques confirm this assignment. Because of the relatively low peak intensities at 1.60 ppm and the spectral complexity in this region, making a similar guess-and-check was difficult.

The relationship between molecular weight and DOSY-derived diffusivity has been exploited before to perform solution structure studies on unknown phosphorus-containing compounds.[12] In this work, the MW-*D* correlation was developed using trialkyl phosphates and used to determine the molecular weight of a phosphorous-containing organolithium compound. To derive an identity for the remaining peak at 1.60 ppm, we applied this technique using VX, HMPA, and EMPA as the calibrating species and determined an estimated molecular weight for the mystery compound at 1.60 ppm.

Figure 7 plots the log(*D*)/log(MW) correlation derived from the diffusivities of VX, EMPA, and HMPA. This curve was then used to predict the molecular weight of the compound at 1.60 ppm using its measured diffusivity of 6.39×10^{-10} m^2/s. This calculation yields log(MW) = 2.408 or MW = 256 g/mol. The fact that this compound's diffusivity is so close to that of the parent VX would lead to the conclusion that their molecular weights should be roughly similar. Considering the possible degradation chemistries resulting from exposure to the cyclen-Zn^{2+} complex and the similarity in molecular weights, we conclude that this compound is EA2192 with a molecular weight of 239.37. The calculated MW agrees well – ca. 7% of the actual molecular weight of EA2192. Comparison of chemical shifts of both the EA2192 CH_3-P doublet and the ^{31}P nucleus from previous research confirms the assignment of this toxic VX byproduct.[17]

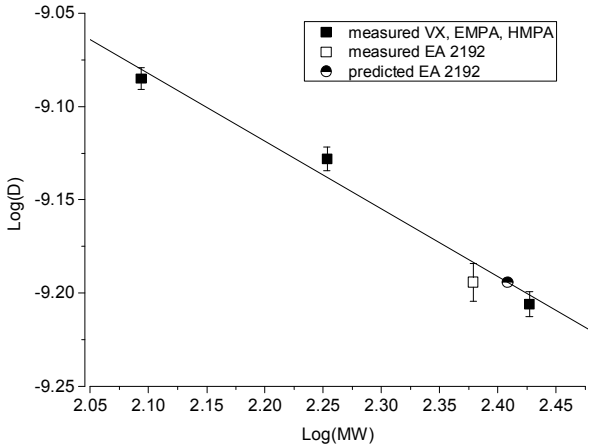

Figure 7. Log(*D*)/log(MW) correlation for VX, EMPA, and HMPA (filled squares). The line is a linear fit to those three points from which a predicted log(MW) for EA2192 is calculated (half-filled circle). The actual MW/measured diffusivity of EA2192 using the molecular weight of the compound is given by the open square.

Conclusions.

A strategy for the simplification of complex proton spectra using ^{31}P-edited HSQC-DOSY sequences has been presented. These experiments were validated using traditional LED-DOSY techniques that lacked HSQC selectivity for samples related to chemical weapons and important not only for OPCW proficiency examinations and inspections but for a wide variety of other organophosphorus containing compounds, particularly pesticides and other toxic environmental pollutants. We demonstrated that HSQC-DOSY sequence could perform the efficient chromatographic-like separation necessary to isolate and identify the essential resonances related to VX degradation by a zinc-cyclen catalyst. More importantly, however, the resultant diffusion data was used to extract the identity of an "unknown" degradation product. Using a log(MW)/log(*D*) correlation and a estimate for the unknown's molecular weight, we suspected this compound to be the well-

documented degradation product EA2192 and were able to confirm this assignment based on literature references and other, more time intensive, multidimensional NMR techniques. This added benefit of the HSQC-DOSY sequence portends the application of this technique to assess the effects various degradation molecules (bleach, peroxides, oximes, etc.) and scenarios for which preferred degradation pathways and products are completely unknown.

Author Information.

*E-mail: mayer22@llnl.gov.

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